APPENDIX TO AMENDMENT OF MAY 13, 2003

Version Showing Changes Marked-Up

In the Claims:

1. (Amended) A pharmaceutical composition [for the treatment or prophylaxis of gastrointestinal disorders], comprising a diaminoalkyl compound and a pharmaceutically acceptable carrier, wherein the pharmaceutical composition attenuates the effect of pathogenic bacterial enterotoxins.

4. (Amended) The composition of claim 1, wherein the gastrointestinal disorders result from an infection by an organism selected from the group consisting of *Shigella* spp. enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli*, uropathogenic *E. coli*, meningitis-causing *E. coli* K-1, *Yersinia pestis, Yersinia pseudotuberculosis, Yersinia enterocolitica,* [*Mycobacterium tuberculosis,*] *Mycobacterium bovis,* [*Mycobacterium avium, Neisseria gonorrhoeae, Neisseria meningitidis,*] *Listeria monocytogenes, Vibrio cholerae, Streptococcus pyogenes, Campylobacter jejuni,* and *Bacteroides fragilis*[, and *Haemophilus influenzae*].

7. (Amended) The method of claim [6] 10, wherein the [mammal] host is a human.

8. (Amended) The method of claim [6] 10, wherein the [gastrointestinal disorders result from an infection by] pathogenic bacterial enterotoxins are produced by a *Shigella* spp.

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Bacterial Pathogenesis

A Molecular Approach

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coli") has been called Shiga-like toxin because it is closely related to the Shiga toxin produced by *Shigella* species, or **verotoxin** because it is toxic for a tissue culture cell line called Vero. An exotoxin produced by C. *perfringens* is called both alpha-toxin and lecithinase. A notorious source of confusion for students first encountering toxin names is the term **enterotoxin.** This is a specific term that denotes protein toxins that cause diarrhea or vomiting, i.e., enteric symptoms, and should not be confused with endotoxin.

Recently, another layer has been added to the nomenclatural nightmare of toxin designations, although this one has the virtue of separating toxins based on the mechanism of action—types I to III toxins (Appendix 1). Normally, the first step in toxin action is binding to the target cell. This binding step may be followed by internalization of a portion of the toxin. **Type I toxins** bind to the host cell surface, but they are not translocated into the host cell. An example of this type of toxin is the **superantigens,** which bind to surface molecules on macrophages and T cells, forcing them into an unnatural interaction in which they produce copious amounts of toxic cytokines (see later section). **Type II toxins** are the ones that act on eukaryotic cell membranes (**phospholipases, pore-forming cytotoxins**) and exert their effect by destroying the integrity of the mammalian cell cytoplasmic membrane. **Type III toxins** are the **A-B toxins,** which have a binding region (B) that recognizes a specific receptor, a translocation region that introduces the A portion into the cell cytoplasm, and an A portion that acts on some intracellular protein.

Exotoxin Structure and Function

A-B TOXINS (TYPE III TOXINS). A-B toxins were the first toxins to be studied in detail at the molecular level, and so they have come to be the paradigm toxins. Only in more recent years have other types of toxins, such as the type I and type II toxins, come in for the same level of attention. Structures of two types of A-B toxins are illustrated in Figure 9–2. The simplest type of A-B toxin is synthesized as a single polypeptide, which has one binding (B) portion and one enzymatic (A) portion. Frequently, the A and B portions of such toxins are separated during processing of the toxin by a proteolytic cleavage event, although the two portions remain connected by disulfide bonds (Figure 9–2). The disulfide bonds are broken when the A portion is internalized by the host cell, and this detachment of the A portion from the B portion is necessary for the A portion to become enzymatically active.

A more complex type of A-B toxin, the compound A-B toxin, has a binding (B) portion composed of multiple subunits, which are identical in some cases but not in others. The enzymatic (A) portion is a separate polypeptide (Figure 9–2). As with the simple A-B toxins, the A portion is attached to the rest of the toxin by disulfide bonds which are broken when the A subunit is internalized by the host cell.

Both the simple and compound A-B toxins bind to and enter host cells as illustrated in Figure 9–3. The B portion binds to a specific host cell surface molecule. Often, the molecule recognized by the B portion is the carbohydrate moiety of a host cell surface glycoprotein or glycolipid, but some B portions bind to proteins. The B portion determines the host cell specificity of the toxin. For example, a toxin whose B portion binds to a glycoprotein that is found only on the surfaces of neurons will function in the body as a neuron-specific toxin even though the A portion has the sort of activity that would enable it to kill other types of host cells if it could gain entry into their cytoplasm.

After the B portion attaches the toxin to the host cell, the A portion is translocated through the host cell membrane into the host cell's cytoplasm. In some cases, the bound toxin is taken up by endocytosis prior to internalization of the A portion into the cytoplasm. Acidification of the endocytic vacuole may play a role in translocation of such toxins by stimulating the separation of A and B portions and internalization of the A portion. For other toxins, endocytosis does not appear to be required; instead, the A portion translocates directly through the host cell's cytoplasmic membrane. Translocation is a complex process that is only beginning to be understood. One model posits that the B portion not only binds the host cell surface but also forms a pore

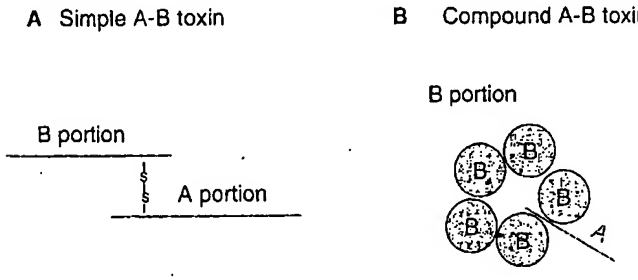

Figure 9–2 Structures of simple and compound A-B toxins. (A) Simple A-B toxins have one A subunit and one B subunit. (B) Compound A-B toxins have one A subunit and multiple B subunits.

Shigella

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General Concepts

Clinical Manifestations

Symptoms of shigellosis include abdominal pain, tenesmus, watery diarrhea, and/or dysentery (multiple scanty, bloody, mucoid stools). Other signs may include abdominal tenderness, fever, vomiting, dehydration, and convulsions.

Structure, Classification, and Antigenic Types

Shigellae are Gram-negative, nonmotile, facultatively anaerobic, non-spore-forming rods. Shigella are differentiated from the closely related Escherichia coli on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylate lysine) and serology. The genus is divided into four serogroups with multiple serotypes: A (S dysenteriae, 12 serotypes); B (S flexneri, 6 serotypes); C (S boydii, 18 serotypes); and D (S sonnei, 1 serotype).

Pathogenesis

Infection is initiated by ingestion of shigellae (usually via fecal-oral contamination). An early symptom, diarrhea (possibly elicited by enterotoxins and/or cytotoxin), may occur as the organisms pass through the small intestine. The hallmarks of shigellosis are bacterial invasion of the colonic epithelium and inflammatory colitis. These are interdependent processes amplified by local release of cytokines and by the infiltration of inflammatory elements. Colitis in the rectosigmoid mucosa, with concomitant malabsorption, results in the characteristic sign of bacillary dysentery: scanty,. unformed stools tinged with blood and mucus.

Host Defenses

Inflammation, copious mucus secretion, and regeneration of the damaged colonic epithelium limit the spread of colitis and promote spontaneous recovery. Serotype-specific immunity is induced by a primary infection, suggesting a protective role of antibody recognizing the lipopolysaccharide (LPS) somatic antigen. Other Shigella antigens include enterotoxins, cytotoxin, and plasmid-encoded proteins that induce bacterial invasion of the epithelium. The protective role of immune responses against these antigens is unclear.

Epidemiology

Shigellosis is endemic in developing countries were sanitation is poor. Typically 10 to 20 percent of enteric

disease, and 50% of the bloody diarrhea or dysentery of young children, can be characterized as shigellosis, and the prevalence of these infections decreases significantly after five years of life. In developed countries, single-source, food or water-borne outbreaks occur sporadically, and pockets of endemic shigellosis can be found in institutions and in remote areas with substandard sanitary facilities.

Diagnosis

Shigellosis can be correctly diagnosed in most patients on the basis of fresh blood in the stool. Neutrophils in fecal smears is also a strongly suggestive sign. Nonetheless, watery, mucoid diarrhea may be the only symptom of many S sonnei infections, and any clinical diagnosis should be confirmed by cultivation of the etiologic agent from stools.

Control

Prevention of fecal-oral transmission is the most effective control strategy. Severe dysentery is treated with ampicillin, trimethoprim-sulfamethoxazole, or, in patients over 17 years old, a 4-fluorquinolone such as ciprofloxacin. Vaccines are not currently available, but some promising candidates are being developed.

INTRODUCTION

Gram-negative, facultative anaerobes of the genus Shigella are the principal agents of bacillary dysentery. This disease differs from profuse watery diarrhea, as is commonly seen in choleraic diarrhea or in enterotoxigenic Escherichia coli diarrhea, in that the dysenteric stool is scant and contains blood, mucus, and inflammatory cells. In some individuals suffering from shigellosis, however, moderate volume diarrhea is a prodrome or the sole manifestation of the infection. Bacillary dysentery constitutes a significant proportion of acute intestinal disease in the children of developing countries, and this infection is a major contributor to stunted growth of these children. Shigellosis also presents a significant risk to travelers from developed countries when visiting in endemic areas, and sporadic food or water-borne outbreaks occur in developed countries.

The pathogenic mechanism of shigellosis is complex, involving a possible enterotoxic and/or cytotoxic diarrheal prodrome, cytokine-mediated inflammation of the colon, and necrosis of the colonic epithelium. The underlying physiological insult that initiates this inflammatory cascade is the invasion of Shigella into the colonic epithelium and the lamina propria. The resulting colitis and ulceration of the mucosa result in bloody, mucoid stools, and/or febrile diarrhea.

Clinical Presentation

Shigellosis has two basic clinical presentations: (1) watery diarrhea associated with vomiting and mild to moderate dehydration, and (2) dysentery characterized by a small volume of bloody, mucoid stools, and abdominal pain (cramps and tenesmus) (Table 22-1). Volunteer challenge studies show that shigellosis can be evoked by an extremely small inoculum (10-100 organisms), and the time of onset of symptoms is somewhat influenced by the size of the challenge. The salient point is that shigellosis is an acute infection with onset of symptoms usually occurring within 24-48 hours of ingestion of the etiologic agent. The average duration of symptoms in untreated adults is 7 days, and the organism may be cultivated from stools for 30 days or longer.

TABLE 22-1 Clinical Characteristics of Shigellosis

Symptom	Approximate Percentage of Patients*		
	S sonnei	S flexneri	S dysenteriae
Watery diarrhea	75	30	30
Stool mucus	50	75	95
Stool blood	10	50	80
Abdominal pain	50	70	65
Vomiting	60	30	40
Fever	5	10	10

* Based on data from Dacca Hospital, Dacca, Bangladesh

The clinical features of shigellosis are summarized in Figure 22-1. Watery diarrhea occurs as a prodrome, or as the sole clinical manifestation, in a majority of patients infected with S sonnei. Diarrhea is often a prodome of the dysentery characterizing infection with other species of Shigella. Recently discovered enterotoxins secreted by S flexneri may contribute to the diarrheal phase as the etiologic agents traverse the small intestine. However, diarrhea is most common in patients who have colitis involving the transverse colon or cecum. These patients evidence net water secretion and impaired absorption in the inflamed colon. In patients experiencing dysentery, involvement is most severe in the distal colon, and the resulting inflammatory colitis is evidenced in frequent scanty stools reflecting the ileocecal fluid flow. Dysentery is also characterized by the daily loss of 200-300 ml of serum protein into the feces. This loss of serum proteins results in depletion of nitrogen stores that exacerbates malnutrition and growth stunting. Depletion of immune factors also increases the risk of concurrent, unrelated infectious disease and contributes to substantial mortality.

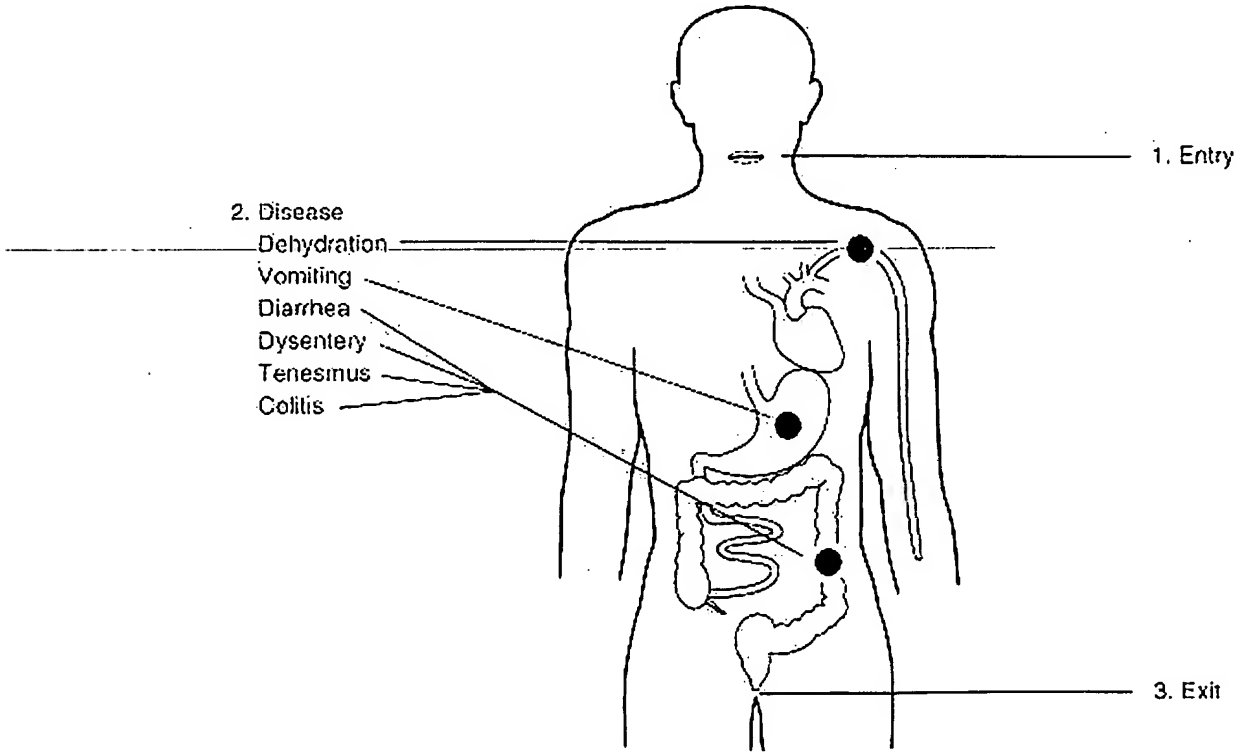

FIGURE 22-1 Pathogenesis of shigellosis in humans.

Possible complications of shigellosis include bacteremia, convulsions and other neurological complications, reactive arthritis, and hemolytic-uremic syndrome. Bacteremia occasionally accompanies S dysenteriae serotype 1 infections in malnourished infants, but this complication is uncommon in otherwise healthy individuals. Convulsions have been reported in up to 25% of Shigella infections involving children under the age of 4 years. Both high fever and a family history of seizures are risk factors for a convulsive episode. Ekiri syndrome, an extremely rare, fatal encephalopathy has also been described in Japanese children with S sonnei or S flexneri infections. Reactive arthritis, a self-limiting sequela of S flexneri infection, occurs in an incidence as high as 2% in individuals expressing the HLA-B27 histocompatibility antigen. Hemolytic-uremic syndrome, characterized by a triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, is a rare complication in children infected with S dysenteriae serotype 1.

Structure, Classification, and Antigenic Types

Organisms of the genus Shigella belong to the tribe Escherichia in the family Enterobacteriaceae. In DNA hybridization studies, Escherichia coli and Shigella species cannot be differentiated on the polynucleotide level; however, the virulence phenotype of the latter species is a distinctive distinguishing feature. Enteroinvasive E coli (EIEC), are very similar to shigellae biochemically and they also evoke diarrhea and/or dysentery. Some EIEC are also serologically related to shigellae. For example, EIEC serotype O124 agglutinates in S dysenteriae serotype 3 antiserum.

The genus Shigella is differentiated into four species: S dysenteriae (serogroup A, consisting of 12 serotypes); S flexneri (serogroup B, consisting of 6 serotypes); S. boydii (serogroup C, consisting of 18 serotypes); and S sonnei (serogroup D, consisting of a single serotype). Serogoups A, B, and C are very similar physiologically while S. sonnei can be differentiated from the other serogroups by positive b-D-galactosidase and ornithine decarboxylase biochemical reactions. The identification of shigellae by species in the clinical laboratory is usually accomplished by slide agglutination using commercially available, absorbed rabbit antisera.

Pathogenesis

Pathology

The rectosigmoidal lesions of shigellosis resemble those of ulcerative colitis. With frequencies indicated in Figure 22-2, there is proximal extension of erythema, edema, loss of vascular pattern, focal hemorrhage, and adherent layers of purulent exudate. Biopsy specimens from affected areas are typically edematous, with capillary congestion, focal hemorrhage, crypt hyperplasia, goblet cell depletion, mononuclear and polymorphonuclear (PMN) cell infiltration, shedding of epithelial cells and erythrocytes, and microulcerations.

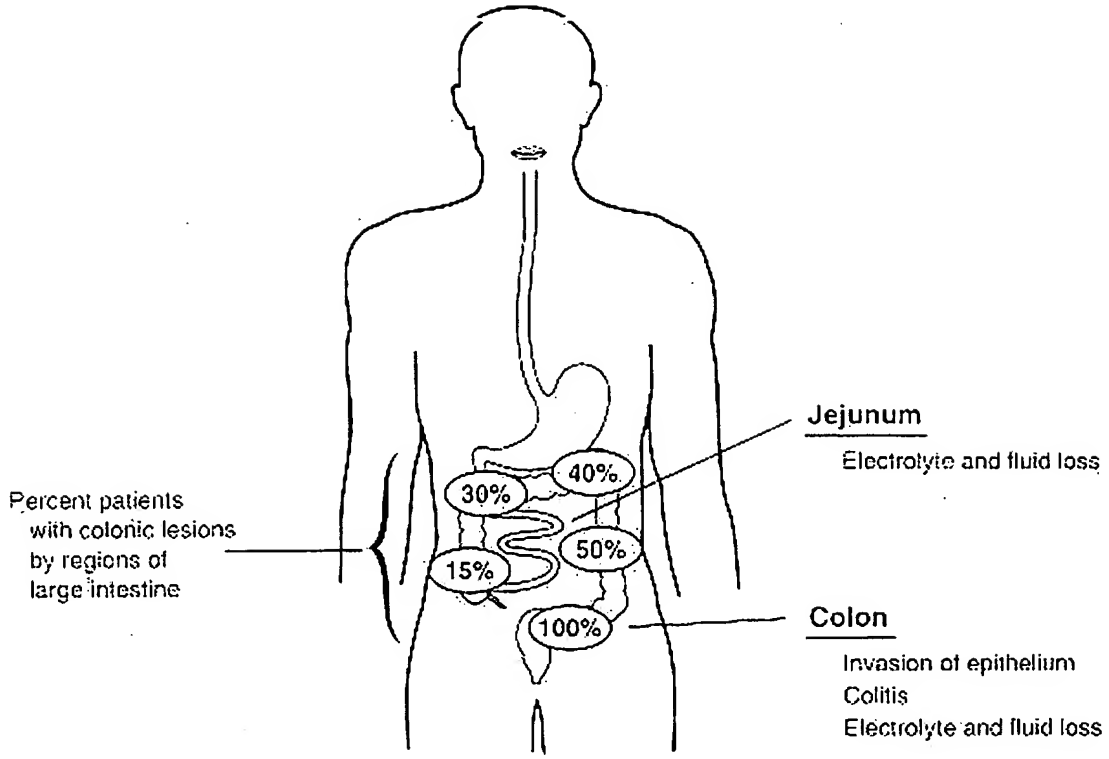

FIGURE 22-2 Gross pathology of shigellosis.

The pathogenic mechanism that underlies these pathological manifestations is diagrammed in Figure 22-3. This cartoon incorporates experimental observations from tissue cultures and from animal models of shigellosis such as rabbit ligated ileal loops injected with virulent organisms. In the latter model, Shigella infection is initiated at the membranous (M) cells that are associated with macroscopic lymphoid follicles (Peyer's patches). Biopsy studies in rhesus monkeys suggest that shigellae also infect microscopic lymphoid follicles of the primate colon. During the early stages of infection, bacteria are transcytosed through the M cells into the subepithelial space. In the subepithelial space, the organisms are phagocytosed by resident macrophages. However, virulent shigellae are not killed and digested in the macrophage phagolysome. The bacteria lyse the phagosome and initiate apoptosis (programmed cell death). During this process, the infected macrophage releases the inflammatory cytokine IL-1, which elicits infiltration of PMN.

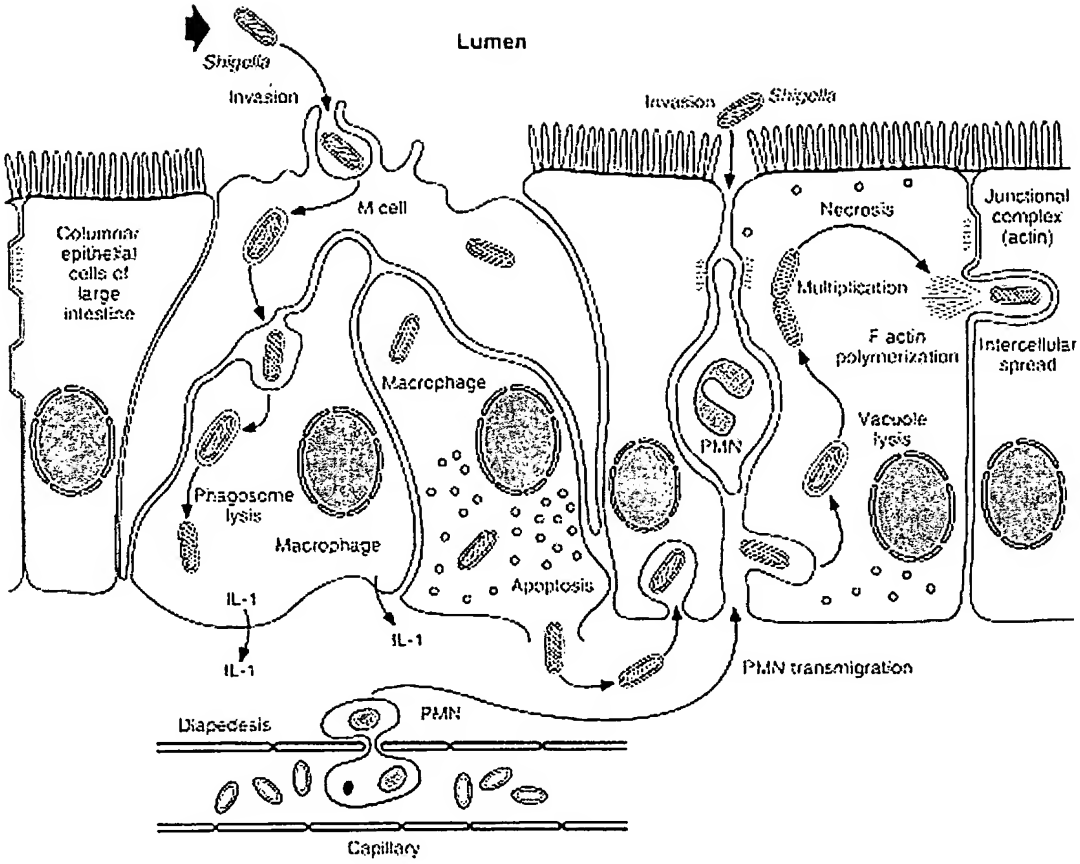

FIGURE 22-3 Histopathology of acute colitis following peroral infection with shigellae. The organisms are initially ingested by membranous (M) cells that are associated with lymphoid microfollicles in the colon. After transcytosis through the M cell, the bacteria are deposited into the subepithelial space where they are phagocytosed by macrophages. The macrophage phagosome is subsequently degraded, and the intracellular shigellae cause release of IL-1 that evokes an influx of polymorphonuclear leukocytes (PMN). Eventually the infected macrophages undergo apoptosis (programmed cell death), and the bacteria are released onto the basolateral surface of adjacent colonic enterocytes. In addition, PMN transmigration through the epithelium disrupts tight junctions, allowing shigellae to migrate into the subepithelial space. The bacteria infect enterocytes by induced endocytosis, and the endocytic vacuoles are subsequently degraded. The intercellular shigellae attach to actin in the enterocyte junctional complex, multiply, and spread to contiguous enterocytes by induced actin polymerization. Ultimately, the infected enterocytes die, and the resulting necrosis of the epithelium, in conjunction with the continuing inflammatory response, constitutes the lesions of shigellosis.

Transmigration of infiltrating PMNs through the tight junctions of local epithelial cells and into the intestinal lumen allows the reverse migration of shigellae from the lumen into the subepithelial spaces. These organisms then infect the columnar epithelial cells by inducing endocytic uptake at the basolateral surface. Immediately after infection of enterocytes, intracellular shigellae lyse endocytic vacuoles and attach to the actin cytoskeleton in the area of the junctional complex. As these organisms multiply within the enterocyte cytoplasm, occasional daughter cells induce polar nucleation of filamentous actin resulting in a "tail" that propels the shigellae into protrusions impinging on contiguous enterocytes. Plasma membranes enveloping the organisms are again lysed, and the organisms are deposited within the contiguous host cell resulting in intercellular bacterial spread.

In summary, shigellosis can be characterized as an acute inflammatory bowel disease initiated by the uptake of only a few organisms into lymphoid follicles. Intracellular replication and intercellular spread leads to an amplified inflammatory cascade at the initial site of entry, and as this inflammation persists and expands, the

infiltration of PMN facilitates the entry of additional bacteria into the epithelium. The inflammatory infiltrate can also cause detachment of sheets of epithelial cells in areas devoid of lymphoid structures or bacterial cells.

Genetics of Virulence

Shigella are exquisitely adapted for reproduction within the colonic epithelium of the human host. Many of the bacterial virulence determinants that mediate the complex interactions between these bacteria and mammalian host cells have been identified by genetic and immunological means. These virulence determinants are encoded by large extra-chromosomal elements (plasmids) that are functionally identical in all Shigella species and in EIEC. A complex of two plasmid-encoded determinants, designated Invasion Plasmid Antigens (Ipa) B and C, is recognized by antibody in the sera of convalescent patients. Ipa proteins are maximally expressed in conditions approximating the intestinal lumen (e.g., bile salts, high osmolarity, and human body temperature), and release of the IpaBC complex is triggered by contact with the mammalian host cell. This complex induces the endocytic uptake of shigellae by M cells, epithelial cells, and macrophages. IpaB also mediates lysis of endocytic vacuoles in epithelial cells or macrophages. In the latter case, Ipa proteins also cause release of the IL-1 cytokine and macrophage apoptosis. Another plasmid-encoded virulence determinant is secreted at the poles of Shigella daughter cells as these organisms multiply within the cytoplasm of infected host cells. This InterCellular Spread (IcsA) protein elicits polymerization of filamentous actin. Formation of this actin tail provides a motive force for shigellae impinging on the plasma membrane of the infected cell. The resulting protrusions deform the plasma membrane of contiguous cells. The IcsB plasmid-encoded protein then lyses the plasma membranes, resulting in intercellular bacterial spread. Biochemical characterization of the interaction between these Shigella virulence determinants and host cell components is a remaining research challenge. Characterizing and enhancing the neutralizing potential of antibody recognizing these protein virulence determinants is also an important research goal.

Toxins

Spent medium from S flexneri or EIEC cultures elicits fluid accumulation in rabbit ligated ileal loops and ion secretion in isolated ileal tissue. Using these assays, enterotoxins designated ShET1 and ShET2 have been identified, and the genetic loci encoding these toxins have been localized to the chromosome and plasmid, respectively. ShET1 is neutralized by convalescent sera from volunteers challenged with S flexneri 2a, suggesting that this toxic moiety is expressed by shigellae growing in the human intestine. The ShET1 locus is present on the chromosome of S flexneri 2a, but it is only occasionally found in other serotypes. In contrast, ShET2 is more widespread and detectable in 80% of shigellae representing all four species. These enterotoxins may elicit the diarrheal prodrome that often precedes bacillary dysentery; however, their role in the disease process remains to be defined by controlled challenge studies using toxin-negative mutants.

S dysenteriae serotype 1 expresses Shiga toxin, an extremely potent, ricin-like, cytotoxin that inhibits protein synthesis in susceptible mammalian cells. This toxin also has enterotoxic activity in rabbit ileal loops, but its role in human diarrhea is unclear, since shigellae apparently express a number of enterotoxins. Experimental infection of rhesus monkeys with S dysenteriae 1, and with a Shiga toxin-negative mutant, suggests that this cytotoxin causes capillary destruction and focal hemorrhage that exacerbates dysentery (see Table 22-1). More importantly, Shiga toxin is associated with the hemolytic-uremic syndrome, a complication of infections with S dysenteriae 1. Closely related toxins are expressed by enterohemorrhagic E coli (EHEC) including the potentially lethal, food-borne O157-H7 serotype.

Host Defense

Shigellae are remarkably infectious enteric pathogenes that can cause disease after the ingestion of as few as 10 organisms. Nonetheless, shigellosis is normally an acute, self-limiting disease that exemplifies the regenerative

capacity of the intestinal epithelium. Shigella virulence probably reflects both the efficient uptake by the follicle associated epithelium (M cells) and the amplifying effect of the inflammatory cascade generated by apoptic macrophages. Tenesmus and evacuation of mucus by intestinal goblet cells may effectively eliminate both extracellular shigellae and infected enterocytes from the intestinal lumen, but this defensive response, in conjunction with PMN infiltration, also constitutes the definitive sign of bacillary dysentery.

In endemic areas, shigellosis is essentially a childhood disease, and the incidence decreases drastically in the indigenous population over 5 years of age. Controlled volunteer challenge studies in North American adults also indicate that prior infection with S flexneri protects against reinfection with the homologous serotype (70% efficacy). Serotype-specific immune protection against shigellosis suggests that antibody recognizing the O-polysaccharide of LPS protects against clinical symptoms. Ingested bovine colostrum containing antibody recognizing the O-polysaccharide of S flexneri 2a passively protects volunteers challenged with the homologous Shigella serotype. These observations have encouraged the development of a number of parenteral and mucosally administered O-polysaccharide vaccines that are currently in safety and/or efficacy trials. These vaccines offer the possibility of effective control of shigellosis independent of the needed improvements in the public health infrastructure of developing countries, but licensure and delivery of practical Shigella vaccines remains a distant prospect.

Epidemiology

Humans are the primary reservoir of Shigella species, with captive subhuman primates as accidental hosts. In developing countries with prevailing conditions of inadequate sanitation and overcrowded housing, the infection is transmitted most often by the excreta of infected individuals via direct fecal-oral contamination. Flies may contribute to spread from feces to food. The most common species, S dysenteriae and S flexneri, are also the most virulent. In developed countries, sporadic common-source outbreaks, predominantly involving S sonnei, are transmitted by uncooked food or contaminated water. The latter outbreaks usually involve semipublic water systems such as those found in camps, trailer parks, and Indian reservations. Direct fecal-oral spread can also occur in institutional environments such as child day-care centers. mental hospitals, and nursing homes. Homosexual men are also at increased risk for direct transmission of Shigella flexneri infections, and chronic, recrudescent illness complicating HIV infection has been reported.

Diagnosis

Clinical

Patients presenting with watery diarrhea and fever should be suspected of having shigellosis. The diarrheal stage of the infection cannot be distinguished clinically from other bacterial, viral, and protozoan infections. Nausea and vomiting can accompany Shigella diarrhea, but these symptoms are also observed during infections with nontyphoidal salmonellae and enterotoxigenic E coli. Bloody, mucoid stools are highly indicative of shigellosis, but the differential diagnosis should include EIEC, Salmonella enteritidis, Yersinia enterocolitica, Campylobacter species, and Entamoeba histolytica. Although blood is common in the stools of patients with amebiasis, it is usually dark brown rather than bright red, as in Shigella infections. Microscopic examination of stool smears from patients with amebiasis should reveal erythrophagocytic trophozoites in the absence of PMN, whereas bacillary dysentery is characterized by sheets of PMN. Sigmoidoscopic examination of a shigellosis patient reveals a diffusely erythematous mucosal surface with small ulcers, whereas amebiasis is characterized by discrete ulcers in the absence of generalized inflammation.

Laboratory

Although clinical signs may evoke the suspicion of shigellosis, diagnosis is dependent upon the isolation and

identification of Shigella from the feces. Positive cultures are most often obtained from blood-tinged plugs of mucus in freshly passed stool specimens obtained during the acute phase of disease. Rectal swabs may also be used to culture shigellae if the specimen is processed rapidly or is deposited in a buffered glycerol saline holding solution. Isolation of shigellae in the clinical laboratory typically involves an initial streaking for isolation on differential/selective media with aerobic incubation to inhibit the growth of the anaerobic normal flora. Commonly used primary isolation media include MacConkey, Hektoen Enteric Agar, and Salmonella-Shigella (SS) Agar. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (Coliforms) from non-lactose fermenters such as shigellae. A liquid enrichment medium (Hajna Gram-negative broth) may also be inoculated with the stool specimen and subcultured onto the selective/differential agarose media after a short growth period. Following overnight incubation of primary isolation media at 37° C, colorless, non-lactose-fermenting colonies are streaked and stabbed into tubed slants of Kligler's Iron Agar or Triple Sugar Iron Agar. In these differential media, Shigella species produce an alkaline slant and an acid butt with no bubbles of gas in the agar. This reaction gives a presumptive identification, and slide agglutination tests with antisera for serogroup and serotype confirm the identification.

Some E coli biotypes of the normal intestinal flora closely resemble Shigella species (i.e. they are nonmotile, delayed lactose fermenters). These coliforms can usually be differentiated from shigellae by the ability to decarboxylate lysine. However, some coliforms cause enteroinvasive disease because they carry the Shigella-like virulence plasmid, and these pathogens are conventionally identified by laborious serological screening for EIEC serotypes. Sensitive and rapid methodology for identification of both EIEC and Shigella species utilizes DNA probes that hybridize with common virulence plasmid genes or DNA primers that amplify plasmid genes by polymerase chain reaction (PCR). Enzyme-linked immunosorbent assay (ELISA) using antiserum or monoclonal antibody recognizing Ipa proteins can also be used to screen stools for enteroinvasive pathogens. These experimental diagnostic techniques are useful for epidemiological studies of enteroinvasive infections, but they are probably too specialized for routine use in the clinical laboratory.

Treatment

Although severe dehydration is uncommon in shigellosis, the first consideration in treating any diarrheal disease is correction of abnormalities that result from isotonic dehydration, metabolic acidosis, and significant potassium loss. The oral rehydration treatment developed by the World Health Organization has proven effective and safe in the treatment of acute diarrhea, provided that the patient is not vomiting or in shock from severe dehydration. In the latter case, intravenous fluid replacement is required until initial fluid and electrolyte losses are corrected. With proper hydration, shigellosis is generally a self-limiting disease, and the decision to prescribe antibiotics is predicated on the severity of disease, the age of the patient, and the likelihood of further transmission of the infection. Effective antibiotic treatment reduces the average duration of illness from approximately 5-7 days to approximately 3 days and also reduces the period of Shigella excretion after symptoms subside. Absorbable drugs such as ampicillin (2 g/day for 5 days) are likely to be effective when the isolate is sensitive. Trimethoprim (8 mg/kg/day) and sulfamethoxazole (40 mg/kg/day) will eradicate sensitive organisms quickly from the intestine, but resistance to this agent is increasing. Ciprofloxacin (1 g/day for 3 days) is effective against multiple drug resistant strains, but this antibiotic is not approved by the United States Food and Drug Administration for use in children less than 17 years of age because there is a theoretical risk of cartilage damage. Opiates, such as paregoric, induce intestinal stasis and may promote bacterial invasion, prolonging the febrile state.

Control

As is the case with other intestinal infections, the most effective methods for controlling shigellosis are provision of safe and abundant water and effective feces disposal. These public health measures are, at best,

long range strategies for control of enteric infections in developing countries. The estimated five million deaths annually attributed to diarrheal disease in these countries, in addition to the malabsorption and growth stunting among survivors, require more immediate and practical approaches. The most effective intervention strategy to minimize morbidity and mortality would involve comprehensive media and personal outreach programs consisting of the following components: (1) education of all residents to actively avoid fecal contamination of food and water and to encourage hand washing after defecation; (2) encourage mothers to breast-feed infants; (3) promote the use of oral rehydration therapy to offset the effects of acute diarrhea; (4) encourage mothers to provide convalescent nutritional care in the form of extra food for children recovering from diarrhea or dysentery.

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DIMETHYL SULFOXIDE

Substance Dimethyl sulfoxide
(DMSO, methyl sulfoxide)
CAS 67-68-5

Formula $(CH_3)_2$ SO

Physical Properties Colorless liquid
bp 189 °C (decomposes), mp 18.5 °C
Miscible with water

Odor Mild garlic odor

Vapor Pressure 0.37 mmHg at 20 °C

Flash Point 95 °C

Autoignition Temperature 215 °C

Toxicity Data LD_{50} oral (rat) 14,500 mg/kg

LD_{50} skin (rabbit) 40,000 mg/kg

LC_{50} inhal (rat) 1600 mg/m^3(4 h)

Major Hazards Freely penetrates skin and may carry dissolved chemicals across the skin.

Toxicity The acute toxicity of DMSO by all routes of exposure is very low. Inhalation of DMSO vapor can cause irritation of the respiratory tract, and at higher concentrations may cause vomiting, chills, headache, and dizziness. The material is only slightly toxic by ingestion and may cause vomiting, abdominal pain, and lethargy. Dimethyl sulfoxide is relatively nontoxic by skin absorption, but can cause itching, scaling, and a transient burning sensation. Dimethyl sulfoxide can increase the tendency for other chemicals to penetrate the skin and so increase their toxic effects. Contact of DMSO liquid with the eyes may cause irritation with redness, pain, and blurred vision.

Chronic exposure to dimethyl sulfoxide can cause damage to the cornea of the eye. Dimethyl sulfoxide has not been found to be carcinogenic or to show reproductive or developmental toxicity in humans.

Flammability and Explosibility Combustible when exposed to heat or flame (NFPA rating = 1). Carbon dioxide or dry chemical extinguishers should be used to fight DMSO fires.

Reactivity and Incompatibility DMSO reacts violently with strong oxidizers, many acyl halides, boron hydrides, and alkali metals. DMSO can form explosive mixtures with metal salts of oxoacids (sodium perchlorate, iron(III) nitrate).

Storage and Handling Dimethyl sulfoxide should be handled in the laboratory using the "basic prudent practices" described in Chapter 5.C.

Accidents In the event of skin contact, immediately wash with soap and water and remove contaminated clothing. In case of eye contact, promptly wash with copious amounts of water for 15 min (lifting upper and lower lids occasionally) and obtain medical attention. If dimethyl sulfoxide is ingested, obtain medical attention immediately. If large amounts of this compound are inhaled, move the person to fresh air and seek medical attention at once.

In the event of a spill, remove all ignition sources, soak up the dimethyl sulfoxide with a spill pillow or absorbent material, place in an appropriate container, and dispose of properly. Respiratory protection may be necessary in the event of a large spill or release in a confined area.

Disposal Excess dimethyl sulfoxide and waste material containing this substance should be placed in an appropriate container, clearly labeled, and handled according to your institution's waste disposal guidelines.

The information in this LCSS has been compiled by a committee of the National Research Council from literature sources and Material Safety Data Sheets and is believed to be accurate as of July 1994. This summary is intended for use by trained laboratory personnel in conjunction with the NRC report *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*. This LCSS presents a concise summary of safety information that should be adequate for most laboratory uses of the title substance, but in some cases it may be advisable to consult more comprehensive references. This information should not be used as a guide to the nonlaboratory use of this chemical.

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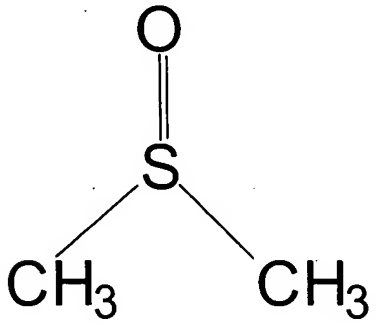
ProductInformation

DIMETHYL SULFOXIDE
Product Code **15,493-8**
Storage Temperature RT
Exact replacement for Product Code D 8779

CAS #: 67-68-5
Synonyms: : A 10846, Deltam, Demeso, Demasorb, Demavet, Demsodrox, Dermasorb, Dimethyl Sulphoxide, Dimexide, Dipirartril-Tropico, DMS-70, DMS-90, DMSO, Dolicur, Domoso, Dromisol, Durasorb, Gamasol 90, Hyadur, Infiltrina, M 176, Methylsulfinylmethane, NSC-763, Rimso-50, Somipront, SQ 9453, Sulfinylbis(methane), Syntexan, Topsym

Product Description



Appearance: Clear, colorless liquid (Note: This product's melting point is near room temperature. Upon shipment, it may arrive as a solid instead of a liquid. DMSO can be remelted at approximately 30°C without affecting the product's performance.)
Molecular formula: C_2H_6SO
Formula weight: 78.13 (anhydrous)
Melting Point: 18.45°C (supercools easily)[1]
Boiling Point: 189°C @760 mm Hg[1]
Specific Gravity: 1.100 @20°C with respect to H_2O @4°C[1]
Autoprotolysis constant = approx. 33 @25°C[2]
Viscosity: 1.1 cp @27°C[1]
Refractive Index: 1.4795 @20°C[1]
Dielectric constant = 45[1]
Purity: Minimum 99.5% (gas chromatography)

DMSO is a highly polar substance with exceptional solvent properties for organic and inorganic chemicals and is widely used as an industrial solvent. DMSO is also used to protect living cells during cold storage.[3] Among its many other uses, DMSO has been used in the oxidation of thiols and disulfides to sulfonic acids.[4]

DMSO is **incompatible** with polysulfone, flexible and rigid PVC tubing and polycarbonate.[5]

It is **moderately** compatible with polystyrene and ECTFE/ETFE.[5]

It is **compatible** with LDPE, HDPE, polypropylene, PPCO polypropylene copolymer polymethylpentene, nylon and teflon FEP.[5]

Preparation Instructions
To prepare a sterile solution, use a teflon or nylon membrane to sterile-filter the DMSO; do not use a cellulose acetate membrane.

DMSO is soluble in water, ethanol, acetone, ether, benzene and chloroform.[1]

DMSO is stable up to 100°C in alkaline, acidic and neutral conditions. At temperatures approaching its boiling point of 189°C, DMSO is stable in neutral or alkaline conditions.[7]

DMSO reacts violently with acyl halides, metal alkoxides, metal oxosalts, perchloric acid and sodium hydroxide.[6]

Storage/Stability
This product should be stored at room temperature and protected from exposure to moisture. DMSO is a very hygroscopic liquid. The purity of the material was essentially unchanged per gas chromatographic analysis. DMSO is thermally stable. It can be heated to 150°C for 24 hours with less than 0.1% loss in purity.[3]

When stored as indicated, DMSO has a shelf-life of two years.

References
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2. Rondinini, S., et al., *Pure & Applied Chem.*, vol. 59, 1693-1702 (1987).

3. *Martindale The Extra Pharmacopoeia*, 29th ed., p. 1426 (1989).
4. Lowe, O.G., *J. Org. Chem.*, vol. 41, 2061 (1976).
5. Nalgene Reference/Chemical Resistance Chart (Nalgene Chemical Company catalog)
6. *Bretherick's Handbook of Reactive Chemical Hazards*, 4th ed., p. 299-303.
7. Supplier's information.

hld 03/03

PRODUCT INFORMATION

McCoy's 5A Medium

(Iwakata and Grace Modification)

CATALOG NO. 51671

JRH BIOSCIENCES
A CSL Company

Description

In 1959, McCoy and his coworkers reported the amino acid requirements for the *in vitro* cultivation of Novikoff Hepatoma cells. These studies were carried out in Basal Medium 5A, originally developed to support Walker carcinoma 256 cells. Modifications to this medium resulting from McCoy's work led to the development of McCoy's 5A Medium.

Formulation

Component	51671
Calcium Chloride, Anhydrous	100.00
Potassium Chloride	400.00
Magnesium Sulfate	97.67
Sodium Chloride	6460.00
Sodium Bicarbonate	2200.00
Sodium Phosphate, Monobasic, Monohydrate	580.00
Bacto-peptone	600.00
Dextrose	3000.00
Glutathione, Reduced	0.50
Phenol Red, Sodium Salt	10.62
L-Alanine	13.36
L-Arginine HCl	42.14
L-Asparagine H_2O	45.03
L-Aspartic Acid	19.97
L-Cysteine HCl H_2O	35.14
L-Glutamic Acid	22.07
L-Glutamine	219.15
Glycine	7.51
L-Histidine HCl H_2O	20.96
L-Hydroxyproline	19.67
L-Isoleucine	39.36
L-Leucine	39.36

Component	51671
L-Lysine HCl	36.54
L-Methionine	14.92
L-Phenylalanine	16.52
L-Proline	17.27
L-Serine	26.28
L-Threonine	17.87
L-Tryptophan	3.06
L-Tyrosine 2Na	26.10
L-Valine	17.57
Ascorbic Acid	0.50
d-Biotin	0.20
D-Ca Pantothenate	0.20
Choline Chloride	5.00
Folic Acid	10.00
myo-Inositol	36.00
Niacin	0.50
Niacinamide	0.50
PABA	1.00
Pyridoxal HCl	0.50
Pyridoxine HCl	0.50
Riboflavin	0.20
Thiamine HCl	0.20
Cyanocobalamin	2.00

All components measured in mg/L

Precautions

Use aseptic technique when handling or supplementing this medium. **For Cell Culture/*In Vitro* Diagnostic Use.** This product is not intended for human or therapeutic use.

Indications of Deterioration

Medium should be clear of particulates and flocculent material. Do not use if liquid medium is cloudy or contains precipitate. Other evidence of deterioration may include color change or degradation of physical or performance characteristics.

An ISO 9001 Certified Quality Management System Company

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Preparation Instructions

Supplements, such as antibiotics, should be added as sterile supplements to the medium. Storage conditions and shelf-life of the supplemented product will be affected by the nature of the supplements. Sterile serum should not be refiltered before or after being added to sterile medium because growth promoting capacity may be reduced upon re-filtration.

Characteristics

Appearance	Red, clear solution
Endotoxin (LAL)	≤ 1.0 EU/mL
Osmolality as supplied	275 - 315 mOsm/kg H_2O
pH at 25 °C as supplied	6.8 - 7.2
Sterility	No microbial growth detected

References

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2. Neuman, R. E. and McCoy, T. A., *PSEBM* (1958) 98:303.
3. Hsu, T. C. and Kellogg, D. S., *JNCI* (1960) 25:221.
4. Iwakata, S. and Grace, J. J., *NY State J Med* (1964) 64:2279.

Ordering & Technical Information

Customer Service

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Issued March 2000 P51671

McCoy's 5A Medium, Modified

Formulation

ATCC®

Catalog No. 30-2007

Inorganic Salts (g/liter)

$CaCl_2$ (anhydrous)	0.10000
$MgSO_4$ (anhydrous)	0.09770
KCl	0.40000
$NaHCO_3$	2.20000
NaCl	6.46000
$NaH_2PO_4 \cdot H_2O$	0.58000

Amino Acids (g/liter)

L-Alanine	0.01336
L-Arginine·HCl	0.04214
L-Asparagine·H_2O	0.04503
L-Aspartic Acid	0.01997
L-Cysteine·HCl·H_2O	0.03514
L-Glutamic Acid	0.02210
L-Glutamine	0.21920
Glycine	0.00750
L-Histidine·HCl·H_2O	0.02096
Hydroxy-L-Proline	0.01970
L-Isoleucine	0.03936
L-Leucine	0.03936
L-Lysine·HCl	0.03654
L-Methionine	0.01492
L-Phenylalanine	0.01652
L-Proline	0.01730
L-Serine	0.02630
L-Threonine	0.01790
L-Tryptophan	0.00310
L-Tyrosine·2Na·2H_2O	0.02612
L-Valine	0.01760

Vitamins (g/liter)

Ascorbic Acid	0.00050
p-Amino Benzoic Acid	0.00100
D-Biotin	0.00020
Choline Chloride	0.00500
Folic Acid	0.01000
myo-Inositol	0.03600
Nicotinamide	0.00050
Nicotinic Acid	0.00050
D-Pantothenic Acid (hemicalcium)	0.00020
Pyridoxine·HCl	0.00100
Riboflavin	0.00020
Thiamine·HCl	0.00020
Vitamin B-12	0.00200

Other (g/liter)

D-Glucose	3.00000
Glutathione (reduced)	0.00050
Bacto-Peptone	0.60000
Phenol Red, Sodium Salt	0.01000

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